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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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EXAMINER	
DUNSTON, JENNIFER ANN	

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1636	

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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/091,177	COME ET AL.	
	Examiner	Art Unit	
	Jennifer Dunston	1636	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *02 November 2007*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-37,39,40,42-63 and 65* is/are pending in the application.

4a) Of the above claim(s) *1-27,47,56-62 and 65* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *28-37,39,40,42-46,48-55 and 63* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *16 July 2007* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Continued Examination Under 37 CFR 1.114

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 11/2/2007 has been entered.

Receipt is acknowledged of an amendment, filed 11/2/2007, in which claims 43, 44 and 63 were amended. Currently, claims 1-37, 39-40, 42-63 and 65 are pending.

Election/Restrictions

Applicant elected Group IV with traverse in the reply filed on 7/20/2005.

Claims 1-27, 47, 56-62 and 65 are withdrawn from consideration as being drawn to a non-elected invention. Applicant timely traversed the restriction (election) requirement in the replies filed on 7/20/2005 and 10/3/2005.

Claims 28-37, 39-40, 42-46, 48-55 and 63 read on the elected invention and are currently under consideration.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person

having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 28-34, 36, 46, 48, 49 and 52-55 are rejected under 35 U.S.C. 103(a) as being unpatentable over Mehta (WO 00/07018, of record; see the entire reference) in view of Keenan et al (Bioorg. Med. Chem. Vol. 6, pages 1309-1335, 1998, of record; see the entire reference). This is a new rejection.

Mehta et al teach a three-hybrid method for identifying the targets such as proteins of biologically active small molecules, where multiple proteins are screened for interactions with any small ligand (e.g., Abstract; page 3, line 19 to page 4, line 2). The method comprises the steps of (i) providing a hybrid ligand that consists essentially of ligand A and ligand B that are linked together, (ii) introducing the hybrid ligand into a sample containing a first expression vector, including DNA encoding the target for ligand A linked to a coding sequence for a first transcriptional module for expression as a first hybrid protein; a second expression vector including sequence from a DNA fragment encoding a polypeptide fused to a second transcriptional module for expression as a second hybrid protein; and a third vector including a reporter gene wherein the expression of the reporter gene is conditioned on the proximity of the

first and second hybrid proteins, (iii) allowing the hybrid molecule to bind to the first hybrid protein through ligand A and to the second hybrid protein through ligand B so as to active the expression of the reporter gene, (iv) identifying samples expressing the reporter gene and characterizing the DNA fragment of the second hybrid protein to determine the protein to which the small molecule is capable of binding (e.g., page 4, lines 3-18; page 25, lines 12-26). The sample environment may be a eukaryotic cell or prokaryotic cell population (e.g., page 7, lines 12-15 and lines 21-29; page 15, lines 1-15). .Mehta et al teach that the hybrid ligand may be introduced into the cell by electroporation or any permeation procedure that is known in the art (e.g., page 15, lines 16-18). Mehta et al teach the screening of DNA sequences from a genomic DNA library, cDNA library or synthetically generated library formed from eukaryotic cells, prokaryotic cells, viruses or formed by automated DNA synthesizer (e.g., paragraph bridging pages 16-17). Mehta et al teach the method where the first ligand (ligand A, or R1 of the claims) binds to the ligand binding domain P1 with high affinity and has a dissociation constant (K_D) of less than 1 μM (e.g., page 17, lines 17-27). Mehta et al teach that ligand A forms a covalent bond with its target and can be a modified aspirin, beta-lactam antibiotic or a mechanism-based enzyme inactivator that binds to the active site of an enzyme and forms a covalent bond with the enzyme (e.g., page 9, lines 13-17; paragraph bridging pages 10-11). The mechanism-based enzyme inactivator compounds inhibit the enzymes listed in Table 2, which includes Protein Kinase C (e.g., page 11, lines 8-11; Table 2). Mehta et al teach the method where ligand A and ligand B are not the same (e.g., page 14, lines 16-18). Further, R2 may be selected from a library of variants (e.g., page14, lines 9-15). Mehta et al teach the method where the first hybrid protein comprises a DNA binding domain and the second hybrid protein comprises an activation domain

(e.g., page 23, line 5 to page 24, line 18; page 26, lines 1-24). Mehta et al teach the method where the reporter gene is green fluorescent protein or LacZ, which codes for beta-galactosidase (e.g., page 17, lines 8-16). Mehta et al teach the confirmation of the dependence of yeast three hybrid ligand interactions on the presence of both fusion proteins and the hybrid ligand by placing yeast cells in a well of a 96 well plate with hybrid ligand only or no hybrid molecule to serve as controls in the assay (e.g. Example 4). Further, Mehta et al teach the screening of libraries to identify numerous proteins that may interact with the hybrid ligand and teach the confirmation of the interactions using the 96-well microtiter assay (e.g. Example 1). Mehta et al teach that ligand A and ligand B may be linked by any linker known in the art (e.g., page 14, lines 19-25). Mehta exemplifies the use of a linker that is a carbon chain of seven carbon atoms (e.g., Figure 4).

Mehta et al do not teach the method where the linker of the hybrid ligand comprising ligand A (R1) and ligand B (R2) has the formula $(CH_2\text{-}O\text{-}CH_2)_n$, where n is an integer from 2 to 5. Mehta et al does not specifically teach that the microtiter plate growth assay is individually conducted on greater than about 10 different positive ligand-binding cell-types identified in the assay.

Keenan et al teach a method comprising the steps of (i) providing a hybrid ligand such as dimerized FK1012 derivative linked by polyethylene linkers, (ii) introducing the hybrid ligand into a population of cells containing a SEAP reporter gene operably linked to ZFHD1 binding sequences, a first chimeric gene encoding a fusion protein containing three FKBP binding domains and a DNA binding domain from ZFHD1, and a second chimeric gene encoding a fusion polypeptide containing three FKBP binding domains and a transcription activation

domain from the NF-κB p65 subunit, and (iii) allowing the hybrid ligand to bind the FKBP binding domains to induce dimerization such that transcription of the SEAP reporter gene is increased, and (iv) identifying positive ligand binding cells by activation of SEAP (e.g. page 1334, Assay for inducible transcriptional activation; Figure 3; Table 1). Keenan et al teach that dimers comprising three or four polyethylene glycol units induced transcriptional activation with potencies only 10- to 20-fold lower than the reference compound 1d (e.g., paragraph bridging pages 1313-1314; Table 1). Thus, Keenan et al teach that the substitution of a 10 atom bisamide linker can be functionally replaced by a polyethylene glycol linker of 3 or 4 units. Increasing the polyethylene glycol units to 5 units results in a decrease in activity to 25% of the reference compound comprising the bisamide linker (e.g., paragraph bridging pages 1313-1314; Table 1).

Because both Mehta et al and Keenan et al teach methods of using a hybrid ligand to activate gene expression in a cell, and because Mehta et al teach it is within the skill of the art to use any known linker to link ligand A and ligand B, it would have been obvious to one of ordinary skill in the art at the time the invention was made to substitute the PEG linker of 3 or 4 units of Kennan et al for the hydrocarbon linker of Mehta et al. Because both linkers have the serve the same purpose and are of about the same length, one would reasonably expect to achieve the predictable result of providing a hybrid ligand capable of functioning in the context of the claimed assay, where the linker is of the formula $(CH_2\text{-}O\text{-}CH_2)_n$, where n is an integer of 3 or 4. Further, it would have been obvious to conduct the assay on greater than 10 ligand-binding cell types because the assay can result in the identification of at least 10 ligand-binding cell types or can be repeated at least 10 times to identify 10 ligand-binding cell types. One would have

been motivated to make such a modification in order to receive the expected benefit of confirming each interaction identified in the screen.

Claims 28-34, 36, 46, 48, 49 and 52-55 are rejected under 35 U.S.C. 103(a) as being unpatentable over Mehta (WO 00/07018, of record; see the entire reference) in view of Bertozzi et al (J. Org. Chem., Vol. 56, pages 4326-4329, 1991, cited as reference CC on the IDS filed 7/20/2005; see the entire reference). This is a new rejection.

Mehta et al teach a three-hybrid method for identifying the targets such as proteins of biologically active small molecules, where multiple proteins are screened for interactions with any small ligand (e.g., Abstract; page 3, line 19 to page 4, line 2). The method comprises the steps of (i) providing a hybrid ligand that consists essentially of ligand A and ligand B that are linked together, (ii) introducing the hybrid ligand into a sample containing a first expression vector, including DNA encoding the target for ligand A linked to a coding sequence for a first transcriptional module for expression as a first hybrid protein; a second expression vector including sequence from a DNA fragment encoding a polypeptide fused to a second transcriptional module for expression as a second hybrid protein; and a third vector including a reporter gene wherein the expression of the reporter gene is conditioned on the proximity of the first and second hybrid proteins, (iii) allowing the hybrid molecule to bind to the first hybrid protein through ligand A and to the second hybrid protein through ligand B so as to active the expression of the reporter gene, (iv) identifying samples expressing the reporter gene and characterizing the DNA fragment of the second hybrid protein to determine the protein to which the small molecule is capable of binding (e.g., page 4, lines 3-18; page 25, lines 12-26). The

sample environment may be a eukaryotic cell or prokaryotic cell population (e.g., page 7, lines 12-15 and lines 21-29; page 15, lines 1-15). Mehta et al teach that the hybrid ligand may be introduced into the cell by electroporation or any permeation procedure that is known in the art (e.g., page 15, lines 16-18). Mehta et al teach the screening of DNA sequences from a genomic DNA library, cDNA library or synthetically generated library formed from eukaryotic cells, prokaryotic cells, viruses or formed by automated DNA synthesizer (e.g., paragraph bridging pages 16-17). Mehta et al teach the method where the first ligand (ligand A, or R1 of the claims) binds to the ligand binding domain P1 with high affinity and has a dissociation constant (K_D) of less than 1 μM (e.g., page 17, lines 17-27). Mehta et al teach that ligand A forms a covalent bond with its target and can be a modified aspirin, beta-lactam antibiotic or a mechanism-based enzyme inactivator that binds to the active site of an enzyme and forms a covalent bond with the enzyme (e.g., page 9, lines 13-17; paragraph bridging pages 10-11). The mechanism-based enzyme inactivator compounds inhibit the enzymes listed in Table 2, which includes Protein Kinase C (e.g., page 11, lines 8-11; Table 2). Mehta et al teach the method where ligand A and ligand B are not the same (e.g., page 14, lines 16-18). Further, R2 may be selected from a library of variants (e.g., page14, lines 9-15). Mehta et al teach the method where the first hybrid protein comprises a DNA binding domain and the second hybrid protein comprises an activation domain (e.g., page 23, line 5 to page 24, line 18; page 26, lines 1-24). Mehta et al teach the method where the reporter gene is green fluorescent protein or LacZ, which codes for beta-galactosidase (e.g., page 17, lines 8-16). Mehta et al teach the confirmation of the dependence of yeast three hybrid ligand interactions on the presence of both fusion proteins and the hybrid ligand by placing yeast cells in a well of a 96 well plate with hybrid ligand only or no hybrid molecule to

serve as controls in the assay (e.g. Example 4). Further, Mehta et al teach the screening of libraries to identify numerous proteins that may interact with the hybrid ligand and teach the confirmation of the interactions using the 96-well microtiter assay (e.g. Example 1). Mehta et al teach that ligand A and ligand B may be linked by any linker known in the art (e.g., page 14, lines 19-25). Mehta exemplifies the use of a linker that is a carbon chain of seven carbon atoms (e.g., Figure 4).

Mehta et al do not teach the method where the linker of the hybrid ligand comprising ligand A (R1) and ligand B (R2) has the formula $(CH_2\text{-}O\text{-}CH_2)_n$, where n is an integer from 2 to 5. Mehta et al does not specifically teach that the microtiter plate growth assay is individually conducted on greater than about 10 different positive ligand-binding cell-types identified in the assay.

At page 4326, Bertozzi et al teach a linker of the following formula:

Bertozzi et al teach that polyethylene glycol derivatives are ideal for the purpose of linking two compounds to make bifunctional molecules for the study of enzymes and receptors, because they are inexpensive, water soluble, and available in a variety of lengths (e.g. page 4326, paragraph bridging column). Bertozzi et al teach that the heterobifunctional linker of the above structure contains a free amine that can be conjugated to biological molecules directly by an amide linkage (or via the corresponding isothiocyanate) and an azide that can be reduced to an amine for conjugation to other molecules (e.g. page 4326, right column). The free amine and azide of the structure of Bertozzi et al is an improvement of the prior art structures, because the Bertozzi

structure is easier to link to biomolecules for the formation of bifunctional compounds (e.g. page 4326). Bertozzi et al exemplify a hybrid ligand comprising mannose linked to fluorescein with a $(CH_2\text{-}O\text{-}CH_2)_3$ linker (e.g. page 4327, left column).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to replace the linker of the hybrid ligand of Mehta et al with the linker taught by Bertozzi et al, because both references teach the use of a linker to link two moieties. Thus, it would have been obvious to one skilled in the art to substitute one linker for another to achieve the predictable result of linking the two moieties for use in the screening assay. Further, it would have been obvious to conduct the assay on greater than 10 ligand-binding cell types because the assay can result in the identification of at least 10 ligand-binding cell types or can be repeated at least 10 times to identify 10 ligand-binding cell types. One would have been motivated to make such a modification in order to receive the expected benefit of confirming each interaction identified in the screen. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Claims 43-45 are rejected under 35 U.S.C. 103(a) as being unpatentable over Johnsson et al (US Patent No. 5,585,245, cited as reference P04 on the IDS filed 4/26/2003; see the entire reference) in view of Licitra et al (PNAS, USA, Vol. 93, pages 12817-12821, 1996, cited in a prior action; see the entire reference) and Bertozzi et al (J. Org. Chem., Vol. 56, pages 4326-4329, 1991, cited as reference CC on the IDS filed 7/20/2005; see the entire reference), as evidenced by Varshavsky et al (PNAS, USA, Vol. 93, pages 12142-12149, 1996, cited in a prior

action; see the entire reference). This rejection was made in the Office action mailed 10/18/2007 and is reiterated below.

Johnsson et al teach a method of identifying the binding between a predetermined member of a specific-binding pair and a previously unidentified member of the specific-binding pair, comprising the steps of (i) providing a first DNA-based expression vector containing an expression cassette encoding a C-terminal subdomain of ubiquitin fused in frame to DNA encoding P1 and to a reporter moiety if P1 does not double as a reporter, (ii) providing a second DNA-based expression vector containing an expression cassette encoding randomly generated genomic or cDNA fragments fused to DNA (P2) encoding the N-terminal subdomain of ubiquitin (Nux), (iii) co-transforming a eukaryotic host cell with the first and second vectors such that the fusion proteins are produced, (iv) detecting cleavage of the fusion protein by the reconstituted ubiquitin moiety (e.g. column 9, lines 28-39; column 12, line 12 to column 13, line 20). Johnsson et al teach the method where the ubiquitin is reconstituted by the interaction of a ligand with P1 and P2 (e.g., Figure 5). Further, Johnsson et al teach that the C-terminal subdomain must bear an amino acid extension (i.e., to form Cub-Z) (e.g. column 6, lines 24-26). Thus, the first expressed fusion protein comprises segments P1, Cub-Z and RM, in an order where Cub-Z is closer to the N-terminus than RM (e.g. column 6, lines 24-26; column 12, line 12 to column 13, line 20; Figure 1D). Johnsson et al teach that the arrangement can also be reversed to have the randomly generated fragment fused to the C-terminal ubiquitin subdomain rather than the N-terminal subdomain (e.g. column 13, lines 15-20). Johnsson et al teach that the system may be used with transmembrane proteins (e.g. column 20, lines 34-45).

Varshavsky is cited only to show that N-end rule degradation operates in all organisms examined, from mammals to fungi and bacteria (e.g. Abstract; page 12147, left column, 1st full paragraph). Thus, the eukaryotic cells taught by Johnsson et al have an N-end rule degradation system.

Johnsson et al do not teach the step of providing a hybrid ligand represented by the general formula R1-Y-R2, wherein Y is a linker with the formula $(CH_2\text{-}O\text{-}CH_2)_n$, where n = 2-5.

Licitra et al teach a yeast three-hybrid assay, wherein a hybrid ligand of Dexamethasone and FK506 is used to screen a cDNA library for proteins capable of binding to FK506 (e.g. page 12818, Three-Hybrid Screen for FK506-Binding Proteins; Figures 1-3). A first hybrid protein comprising a DNA-binding domain and receptor for Dexamethasone (P1) was provided, and a second hybrid ligand comprising a potential receptor for FK506 (P2) and a transactivation domain was provided (e.g. Figures 2-4). Licitra et al teach the identification of positive interactions using the LacZ reporter gene (e.g. Figure 3). Licitra et al teach that the yeast three-hybrid assay has advantages over classical methods for identifying receptors for small ligand in that the system allows the direct isolation and identification of cDNAs encoding receptors and the system easily allows one to manipulate a large number of yeast colonies to study the structure-function relationship of ligand receptor interaction (e.g. page 12820, right column, last full paragraph). Further, Licitra et al suggest the use of other two- and three-hybrid systems that would allow the utility of the system to be expanded to other types of proteins such as membrane proteins (e.g. page 12820, right column, 1st full paragraph).

At page 4326, Bertozzi et al teach a linker of the following formula:

Bertozzi et al teach that polyethylene glycol derivatives are ideal for the purpose of linking two compounds to make bifunctional molecules for the study of enzymes and receptors, because they are inexpensive, water soluble, and available in a variety of lengths (e.g. page 4326, paragraph bridging column). Bertozzi et al teach that the heterobifunctional linker of the above structure contains a free amine that can be conjugated to biological molecules directly by an amide linkage (or via the corresponding isothiocyanate) and an azide that can be reduced to an amine for conjugation to other molecules (e.g. page 4326, right column). The free amine and azide of the structure of Bertozzi et al is an improvement of the prior art structures, because the Bertozzi structure is easier to link to biomolecules for the formation of bifunctional compounds (e.g. page 4326). Bertozzi et al exemplify a hybrid ligand comprising mannose linked to fluorescein with a $(CH_2\text{-}O\text{-}CH_2)_3$ linker (e.g. page 4327, left column).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the two-hybrid method of Johnsson et al to include the hybrid ligand, P1 and P2 portions taught by Licitra et al because Licitra suggest the use of other two- or three-hybrid systems to expand the utility of the assay comprising the hybrid ligand and Johnsson et al teaches a version of a three-hybrid method. Further, it would have been obvious to one of ordinary skill in the art at the time the invention was made to replace the linker of the hybrid ligand of Licitra et al with the linker taught by Bertozzi et al, because both references teach the use of a linker to link two moieties. Thus, it would have been obvious to one skilled in the art to

substitute one linker for another to achieve the predictable result of linking the two moieties for use in the screening assay.

One would have been motivated to make such a modification in order to receive the expected benefit of being able to identify membrane proteins capable of interacting with FK506 as suggested by Licitra and as taught by Johnsson et al. This modification would expand the utility of the hybrid ligand screening assay. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Claims 28-34, 36, 46, 48-50, 52 and 53 are rejected under 35 U.S.C. 103(a) as being unpatentable over Liu et al (US Patent No. 5,928,868, cited in a prior action; see the entire reference) in view of Bertozzi et al (J. Org. Chem., Vol. 56, pages 4326-4329, 1991, cited as reference CC on the IDS filed 7/20/2005; see the entire reference). This rejection was made in the Office action mailed 10/18/2007 and is reiterated below.

Liu et al teach a screening assay for identifying a polypeptide sequence that binds to a user-specified ligand, comprising the steps of (i) providing a hybrid ligand having the general formula A-L-B (or R1-L-R2), where A is a first ligand and B is a user-specified ligand different from A, (ii) introducing the hybrid ligand into a sample containing a functional transcriptional and translational apparatus (for example, a whole cell) that includes vectors a encoding a hybrid protein including a transcription module and a target module for binding ligand A (P1) or for binding ligand B (P2) (target proteins #1 and #2), (iii) once the three hybrid complex comprising the hybrid ligand, first fusion protein and second fusion protein is formed, transcriptional

activation of a reporter gene occurs, and (iv) retrieving the plasmid encoding the fusion protein capable of binding to B and sequencing the plasmid (e.g. column 5. line 55 to column 8, line 46; column 11, lines 25-32; Figure 2). Liu et al teach that one of the vectors capable of binding the ligand contains a DNA binding domain and the other contains a transcription activation domain (e.g. column 7, lines 24-59; Figures 1-3). Liu et al teach that the nucleic acid sequence encoding the ligand B binding domain polypeptide is from random DNA sequences of a size that is capable of encoding a yet undetermined target protein, where the random sequences are derived from a genomic DNA library, cDNA library or synthetically generated library formed from eukaryotic cells, prokaryotic cells, viruses, or formed by an automated DNA synthesizer (e.g. paragraph bridging columns 7-8). With regard to the affinity of the hybrid ligand A to P1, Liu et al teach binding affinities including a Kd below 10^{-6}, 10^{-7}, 10^{-8} or 10^{-9} (e.g. column 8, lines 31-46). Liu et al teach that ligand A may be selected based upon a strong binding affinity for a target encoded by a fusion gene; the binding affinity must necessarily be measured if this determination is made (e.g. column 8, lines 31-46). Liu et al teach that A may form a covalent bond with P1 if a suicide inhibitor is used, for example beta-lactamase as P1 can covalently bind suicide inhibitors used as ligand A, including beta-lactam antibiotics (e.g. paragraph bridging columns 5-6). With regard to the reporter gene, Liu et al teach the use of LacZ, and GFP (e.g. column 8, lines 17-30; Figures 1-3). With regard to ligand B, Liu et al teach that the ligand may be selected from FK506, peptide libraries, nucleic acid libraries, polysaccharide libraries, and small organic molecules (e.g. column 6, lines 14-26). Liu et al teach the use of control experiments containing hybrid ligand only (without target proteins #1 and #2) to determine if the effects of the hybrid ligand are independent of trimeric complex formation (e.g. Example 5). Liu

et al teach the use of control experiments to confirm that reporter gene activation does not occur in the presence of the two target proteins in the absence of the hybrid ligand (e.g. column 11, lines 11-32). Liu et al teach that ligand A and ligand B may be covalently linked by any of the methods known in the art (e.g. column 6, lines 27-37).

Liu et al do not teach the linker between ligand A (or R1) and B (or R2) with the formula $(CH_2\text{-}O\text{-}CH_2)_n$, where n = 2-5.

At page 4326, Bertozzi et al teach a linker of the following formula:

Bertozzi et al teach that polyethylene glycol derivatives are ideal for the purpose of linking two compounds to make bifunctional molecules for the study of enzymes and receptors, because they are inexpensive, water soluble, and available in a variety of lengths (e.g. page 4326, paragraph bridging column). Bertozzi et al teach that the heterobifunctional linker of the above structure contains a free amine that can be conjugated to biological molecules directly by an amide linkage (or via the corresponding isothiocyanate) and an azide that can be reduced to an amine for conjugation to other molecules (e.g. page 4326, right column). The free amine and azide of the structure of Bertozzi et al is an improvement of the prior art structures, because the Bertozzi structure is easier to link to biomolecules for the formation of bifunctional compounds (e.g. page 4326). Bertozzi et al exemplify a hybrid ligand comprising mannose linked to fluoescein with a $(CH_2\text{-}O\text{-}CH_2)_3$ linker (e.g. page 4327, left column).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the hybrid ligand of the three-hybrid method of Liu et al to include the

linker comprising a free amine and azide of Bertozzi et al because Liu et al teach it is within the ordinary skill in the art to use any method known in the art to link ligands A and B to form a hybrid molecule and Bertozzi et al teach the use of the linker to form a hybrid ligand.

One would have been motivated to make such a modification in order to receive the expected benefit of easily linking ligands A and B as taught by Bertozzi et al. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Claims 28-36, 46, 48-50, 52 and 53 are rejected under 35 U.S.C. 103(a) as being unpatentable over Liu et al (US Patent No. 5,928,868, cited in a prior action; see the entire reference) in view of Bertozzi et al (J. Org. Chem., Vol. 56, pages 4326-4329, 1991, cited as reference CC on the IDS filed 7/20/2005; see the entire reference) and Lin et al (Journal of the American Chemical Society, Vol. 122, pages 4247-4248 and supporting pages S1-S12, published online 4/13/2000, cited in a prior action; see the entire reference). This rejection was made in the Office action mailed 10/18/2007 and is reiterated below.

The teachings of Liu are described above and applied as before.

Liu et al do not teach the linker between ligand A (or R1) and B (or R2) with the formula $(CH_2\text{-}O\text{-}CH_2)_n$, where n = 2-5. Liu et al do not teach the three-hybrid method where A (or R1) is methotrexate.

At page 4326, Bertozzi et al teach a linker of the following formula:

Bertozzi et al teach that polyethylene glycol derivatives are ideal for the purpose of linking two compounds to make bifunctional molecules for the study of enzymes and receptors, because they are inexpensive, water soluble, and available in a variety of lengths (e.g. page 4326, paragraph bridging column). Bertozzi et al teach that the heterobifunctional linker of the above structure contains a free amine that can be conjugated to biological molecules directly by an amide linkage (or via the corresponding isothiocyanate) and an azide that can be reduced to an amine for conjugation to other molecules (e.g. page 4326, right column). The free amine and azide of the structure of Bertozzi et al is an improvement of the prior art structures, because the Bertozzi structure is easier to link to biomolecules for the formation of bifunctional compounds (e.g. page 4326). Bertozzi et al exemplify a hybrid ligand comprising mannose linked to fluoescein with a $(CH_2\text{-}O\text{-}CH_2)_3$ linker (e.g. page 4327, left column).

Lin et al teach a method of identifying a polypeptide sequence that binds to a user-specified ligand, comprising the steps of (i) providing a hybrid ligand comprising methotrexate linked to dexamethasone through a linker region, (ii) introducing the hybrid ligand into yeast cells comprising a LacZ reporter gene operably linked to a LexA binding site, a first chimeric gene encoding a fusion polypeptide of LexA and DHFR, a second chimeric gene encoding a fusion protein of GR and B42, (iii) allowing the hybrid ligand to bind the first and second fusion proteins to result in an increase in the level of the transcription of the reporter gene, (iv) identifying a positive ligand binding cell by detecting blue colonies of yeast grown on X-gal containing plates, and (v) identifying the nucleic acid sequence of the second chimeric gene (e.g.

page 4248, left column; Figures 1 and 2; Scheme 1; page S6). Further, Lin et al teach the assay where one of the fusion proteins is deleted to detect the effect of the hybrid ligand independent of the formation of the trimeric complex of the two fusion proteins and the hybrid ligand (e.g. page 4248, left column, last paragraph). Moreover, Lin et al teach the assay in the absence of the hybrid ligand to confirm that the transcription of the reporter gene is dependent on the presence of the hybrid ligand and fusion proteins (e.g. Figure 2). Lin et al teach that methotrexate can be modified readily without disrupting receptor binding, is commercially available, and has a picomolar affinity for DHFR (e.g. page 4247, right column, 1st paragraph).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the hybrid ligand of the three-hybrid method of Liu et al to include the linker comprising a free amine and azide of Bertozzi et al because Liu et al teach it is within the ordinary skill in the art to use any method known in the art to link ligands A and B to form a hybrid molecule and Bertozzi et al teach the use of the linker to form a hybrid ligand. Further, it would have been obvious at the time the invention was made to modify the hybrid ligand of Liu et al to include methotrexate as A (or R1), because Liu et al teach that A can be varied and Lin et al teach the use of methotrexate in a three-hybrid assay.

One would have been motivated to make such a modification in order to receive the expected benefit of easily linking ligands A and B as taught by Bertozzi et al. Further, one would have been motivated to use methotrexate as R1, because Lin et al teach that methotrexate can be modified readily without disrupting receptor binding, is commercially available, and has a picomolar affinity for DHFR. Based upon the teachings of the cited references, the high skill of

one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Claims 28-34, 36, 46 and 48-53 are rejected under 35 U.S.C. 103(a) as being unpatentable over Liu et al (US Patent No. 5,928,868, cited in a prior action; see the entire reference) in view of Bertozzi et al (J. Org. Chem., Vol. 56, pages 4326-4329, 1991, cited as reference CC on the IDS filed 7/20/2005; see the entire reference) and Karlsson et al (US Patent No. 6,143,574, cited in a prior action; see the entire reference). This rejection was made in the Office action mailed 10/18/2007 and is reiterated below.

The teachings of Liu are described above and applied as before.

Liu et al do not teach the linker between ligand A (or R1) and B (or R2) with the formula $(CH_2\text{-}O\text{-}CH_2)_n$, where n = 2-5. Liu et al do not teach the use of plasmon resonance to determine the binding affinity of A to a fusion protein.

At page 4326, Bertozzi et al teach a linker of the following formula:

Bertozzi et al teach that polyethylene glycol derivatives are ideal for the purpose of linking two compounds to make bifunctional molecules for the study of enzymes and receptors, because they are inexpensive, water soluble, and available in a variety of lengths (e.g. page 4326, paragraph bridging column). Bertozzi et al teach that the heterobifunctional linker of the above structure contains a free amine that can be conjugated to biological molecules directly by an amide linkage (or via the corresponding isothiocyanate) and an azide that can be reduced to an amine for

conjugation to other molecules (e.g. page 4326, right column). The free amine and azide of the structure of Bertozzi et al is an improvement of the prior art structures, because the Bertozzi structure is easier to link to biomolecules for the formation of bifunctional compounds (e.g. page 4326). Bertozzi et al exemplify a hybrid ligand comprising mannose linked to fluoescein with a $(CH_2\text{-}O\text{-}CH_2)_3$ linker (e.g. page 4327, left column).

Karlsson et al teach that the BIAcore instrument uses the phenomenon of surface plasmon resonance to study the binding of analytes to receptors immobilized on a sensor chip to allow the affinity and kinetic analysis of interactions between soluble analytes and their immobilized binding partners to be determined (e.g. column 1, lines 11-45). Karlsson et al teach that affinity and kinetic properties for the solution interaction between an analyte and a binding partner can be determined by the following steps: (i) mixing the analyte with an immobilized binding partner (e.g. column 2, lines 3-15; column 3, lines 17-20). Karlsson et al teach that the method provides the ability to not only determine the true affinity properties but also true kinetic properties for the solution interaction between an analyte and binding partner therefore to thereby among other things be permitted a wider choice of reaction partners than in solid phase interactions and avoid immobilization artifacts (e.g. column 1, lines 59-65).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the hybrid ligand of the three-hybrid method of Liu et al to include the linker comprising a free amine and azide of Bertozzi et al because Liu et al teach it is within the ordinary skill in the art to use any method known in the art to link ligands A and B to form a hybrid molecule and Bertozzi et al teach the use of the linker to form a hybrid ligand. Further, it would have been obvious at the time the invention was made to include the use of plasmon

resonance to determine the binding affinity of A to P1, because Liu et al teach it is within the skill of the art to select A and P1 based upon binding affinity and Karlsson et al teach a method of determining binding affinity using plasmon resonance.

One would have been motivated to make such a modification in order to receive the expected benefit of easily linking ligands A and B as taught by Bertozzi et al. Further, one would have been motivated to use the plasmon resonance method of Karlsson et al in order to receive the expected benefit of providing the ability to not only determine the true affinity properties but also true kinetic properties for the solution interaction between an analyte and binding partner therefore to thereby among other things be permitted a wider choice of reaction partners than in solid phase interactions and avoid immobilization artifacts. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Claims 28-34, 36, 46, 48-50, 52, 53 and 63 are rejected under 35 U.S.C. 103(a) as being unpatentable over Liu et al (US Patent No. 5,928,868, cited in a prior action; see the entire reference) in view of Bertozzi et al (J. Org. Chem., Vol. 56, pages 4326-4329, 1991, cited as reference CC on the IDS filed 7/20/2005; see the entire reference) and Licitra et al (PNAS, USA, Vol. 93, pages 12817-12821, 1996, cited in a prior action; see the entire reference). This rejection was made in the Office action mailed 10/18/2007 and is reiterated below.

The teachings of Liu are described above and applied as before.

Liu et al do not teach the linker between ligand A (or R1) and B (or R2) with the formula $(CH_2\text{-}O\text{-}CH_2)_n$, where n = 2-5. Liu et al do not teach the step of providing access to data, nucleic acids or peptides obtained from the identification of polypeptide binding to a hybrid ligand.

At page 4326, Bertozzi et al teach a linker of the following formula:

Bertozzi et al teach that polyethylene glycol derivatives are ideal for the purpose of linking two compounds to make bifunctional molecules for the study of enzymes and receptors, because they are inexpensive, water soluble, and available in a variety of lengths (e.g. page 4326, paragraph bridging column). Bertozzi et al teach that the heterobifunctional linker of the above structure contains a free amine that can be conjugated to biological molecules directly by an amide linkage (or via the corresponding isothiocyanate) and an azide that can be reduced to an amine for conjugation to other molecules (e.g. page 4326, right column). The free amine and azide of the structure of Bertozzi et al is an improvement of the prior art structures, because the Bertozzi structure is easier to link to biomolecules for the formation of bifunctional compounds (e.g. page 4326). Bertozzi et al exemplify a hybrid ligand comprising mannose linked to fluoescein with a $(CH_2\text{-}O\text{-}CH_2)_3$ linker (e.g. page 4327, left column).

Licitra et al teach a yeast three-hybrid assay, wherein a hybrid ligand of Dexamethasone and FK506 is used to screen a cDNA library for proteins capable of binding to FK506 (e.g. page 12818, Three-Hybrid Screen for FK506-Binding Proteins; Figures 1-3). Licitra et al teach the identification of positive interactions using the LacZ reporter gene and disclose the data in the publication (e.g. Figure 3). Licitra et al teach that the yeast three-hybrid assay has advantages

over classical methods for identifying receptors for small ligand in that the system allows the direct isolation and identification of cDNAs encoding receptors and the system easily allows one to manipulate a large number of yeast colonies to study the structure-function relationship of ligand receptor interaction (e.g. page 12820, right column, last full paragraph).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the hybrid ligand of the three-hybrid method of Liu et al to include the linker comprising a free amine and azide of Bertozzi et al because Liu et al teach it is within the ordinary skill in the art to use any method known in the art to link ligands A and B to form a hybrid molecule and Bertozzi et al teach the use of the linker to form a hybrid ligand. Further, it would have been obvious to provide the public access to the data through publication as taught by Licitra et al, because Liu et al teach a three-hybrid assay and Licitra teach a three-hybrid assay and provide the data obtained from the assay.

One would have been motivated to make such a modification in order to receive the expected benefit of easily linking ligands A and B as taught by Bertozzi et al. One would have been motivated to publish the data obtained from such an assay to be able to communicate the findings to peers in the form of a publication. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Claims 28-34, 36-37, 39-40, 42, 46, 48-50, 52 and 53 are rejected under 35 U.S.C. 103(a) as being unpatentable over Liu et al (US Patent No. 5,928,868, cited in a prior action; see the entire reference) in view of Bertozzi et al (J. Org. Chem., Vol. 56, pages 4326-4329, 1991, cited

as reference CC on the IDS filed 7/20/2005; see the entire reference) and Zaharevitz et al (Cancer Research, Vol. 59, pages 2566-2569, cited in a prior action, 1999; see the entire reference). This rejection was made in the Office action mailed 10/18/2007 and is reiterated below.

The teachings of Liu et al are described above and applied as before.

Liu et al do not teach the linker between ligand A (or R1) and B (or R2) with the formula $(CH_2\text{-}O\text{-}CH_2)_n$, where n = 2-5. Liu et al do not teach the method where ligand B is a cyclin dependent kinase inhibitor of Table 2.

At page 4326, Bertozzi et al teach a linker of the following formula:

Bertozzi et al teach that polyethylene glycol derivatives are ideal for the purpose of linking two compounds to make bifunctional molecules for the study of enzymes and receptors, because they are inexpensive, water soluble, and available in a variety of lengths (e.g. page 4326, paragraph bridging column). Bertozzi et al teach that the heterobifunctional linker of the above structure contains a free amine that can be conjugated to biological molecules directly by an amide linkage (or via the corresponding isothiocyanate) and an azide that can be reduced to an amine for conjugation to other molecules (e.g. page 4326, right column). The free amine and azide of the structure of Bertozzi et al is an improvement of the prior art structures, because the Bertozzi structure is easier to link to biomolecules for the formation of bifunctional compounds (e.g. page 4326). Bertozzi et al exemplify a hybrid ligand comprising mannose linked to fluoescein with a $(CH_2\text{-}O\text{-}CH_2)_3$ linker (e.g. page 4327, left column).

Zaharevitz et al teach a small molecule cyclin dependent kinase inhibitor found on page 1 of instant Table 2 (e.g. Figures 1 and 4). Zaharevitz et al teach that these compounds are novel and are able to interact with a subset of CDKs (e.g. paragraph bridging pages 2568-2569). Further, Zaharevitz et al teach that the disclosed compounds are useful as a tool for exploring the structural bases and pharmacological significance of various kinase specificites.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the hybrid ligand of the three-hybrid method of Liu et al to include the linker comprising a free amine and azide of Bertozzi et al because Liu et al teach it is within the ordinary skill in the art to use any method known in the art to link ligands A and B to form a hybrid molecule and Bertozzi et al teach the use of the linker to form a hybrid ligand. Further, it would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the hybrid ligand of the three-hybrid method of Liu et al to include the CDK inhibitors disclosed by Zaharevitz et al as ligand B, because Liu et al teach that ligand B may be selected from a small molecule library and Zaharevitz et al teach that the kinase inhibitor is a small molecule.

One would have been motivated to make such a modification in order to receive the expected benefit of easily linking ligands A and B as taught by Bertozzi et al. Further, one would have been motivated to use the kinase inhibitors of Zaharevitz et al as ligand B to be able to screen for other kinases capable of binding the inhibitors to further characterize the kinase specificities of the inhibitors. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Claims 28-34, 36, 46, 48-50, 52 and 53 are rejected under 35 U.S.C. 103(a) as being unpatentable over Liu et al (US Patent No. 5,928,868, cited in a prior action; see the entire reference) in view of Holt et al (WO 96/06097, cited as reference AD on the IDS filed 4/28/2003; see the entire reference). This rejection was made in the Office action mailed 10/18/2007 and is reiterated below.

The teachings of Liu are described above and applied as before.

Liu et al do not teach the linker between ligand A (or R1) and B (or R2) with the formula $(CH_2\text{-}O\text{-}CH_2)_n$, where n = 2-5.

Holt et al teach the formation of heterodimers of immunophilin ligand moieties (e.g. FK506 and derivatives thereof) (e.g. page 1; page 6, lines 12-17). Holt et al exemplify structures linked by polyethylene linkers of the formula $(CH_2\text{-}O\text{-}CH_2)_n$, where n = 1, 2, 3, 4 (e.g. pages 14-15). Holt et al teach the use of the homodimeric ligands comprising the abovementioned linker where n = 2 or 3 in an cell-based transfection assay where a trimeric complex comprising the homodimeric ligand, a fusion protein comprising three copies of FKBP12 fused to a Gal4 DNA binding domain, and a fusion protein comprising three copies of FKBP12 fused to a VP16 activation domain was detected by the production of the reporter product, secreted alkaline phosphatase (e.g. pages 48-49). Holt et al teach that multimerizers vary somewhat in their observed activity, depending upon the particular chimeric proteins and other components of the system and recommend that the practitioner select multimerizers based upon their performance in the particular system of interest (e.g. page 48 lines 26-30).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the hybrid ligand in the three-hybrid assay to include the $(CH_2\text{-}O\text{-}CH_2)_n$ linker of Holt et al because Liu et al teach it is within the ordinary skill in the art to use any linker known in the art and Holt et al teach linkers for making homodimeric or heterodimeric ligands capable of forming a trimeric complex in three hybrid assay.

One would have been motivated to make such a modification in order to receive the expected benefit of being able to vary the linker of the hybrid ligand to determine which ligand performs best in the three-hybrid system as taught by Holt et al. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Response to Arguments - 35 USC § 103

The rejection of claims 54-55 under 35 U.S.C. 103(a) as being unpatentable over Keenan et al, as evidenced by Amara et al, in view of Mehta et al has been withdrawn. A new rejection is presented above over Mehta et al in view of Keenan et al.

Applicant's arguments filed 11/2/2007 have been fully considered as they apply to the new rejections presented above and the rejections of record, but they are not persuasive.

The response asserts that Keenan teaches away from the claimed method. The response states that Keenan "highlights the 'dramatic difference' in effectiveness, supporting the idea that the limited activity of the PEG linkers is *materially low* compared to the other linkers tested. Thus, based on this result, the 'predictable outcome' as viewed by one of skill in the art, is that a

ligand with the PEG linker may have minimal (if any) activity in the claimed method." The response does not specifically point to the portions of Keenan et al that demonstrate a "dramatic difference" in effectiveness or "materially low" activity. Keenan et al teach that the PEG linkers have minimal (if any) activity in stable transfection assays (e.g., Table 1). In contrast, Keenan et al state, "In the transient transcription assay, **1o**, **1p**, and **1q** induced transcriptional activation with potencies only 10- to 20-fold lower than **1d** while the longer-linked compound **1r** was able only to achieve one-fourth the level of expression as **1d**." See page 1313, right column, last paragraph. Ligands 1p, 1q and 1r had 85%, 60% and 25% the activity of the reference compound, compound 1d. Thus, these ligands successfully activate transcription in the transient transfection based assay. A known or obvious composition does not become patentable simply because it has been described as somewhat inferior to some other product for the same use." *In re Gurley*, 27 F.3d 551, 554, 31 USPQ2d 1130, 1132 (Fed. Cir. 1994). While Keenan et al teach away from using the ligands in a stable transfection assay, the performance of compounds 1p and 1q in the transient transfection assay does not discredit the use of these linkers in the synthesis of a hybrid ligand for transient transfection assays.

Applicant asserts that Applicants have found the claimed linkers have unexpected properties. Applicant asserts that the unexpected property of the PEG linker is that it actually increases the cellular uptake of the hybrid ligands, as shown in Figures 6 and 7 and Example 7 of the specification.

Example 7 of the specification provides a side by side comparison of Mtx-mdbt-Dex (Lin et al., J. Am. Chem. Soc. 2000, 122:4247-8) and Mtx-(ethyleneglycol)$_3$-Dex (GPC 285937). Each of the compounds was added to medium into which yeast cells were added. GPC 285937

allowed growth of the yeast cells at a concentration between 25 to 400 μM showing optimum growth at 100 μM. In contrast, Mtx-mdbt-Dex showed severe precipitation in the medium, which was hypothesized to cause the compound to be less bio-available and hence the growth of the yeast cells was impaired relative to GPC 285937 (e.g., page 128). In a halo assay, where 1 μl of a 1 mM solution of GPC 285937 or Mtx-mdbt-Dex dissolved in DMSO was spotted in the center of petri dishes containing yeast cells. The growth halo for yeast cells receiving Mtx-mdbt-Dex was much smaller than that of GPC 285937 (e.g., page 129; Figure 6a and 6b). Under conditions appropriate to library screening of yeast cell, yeast cells were plated on plates containing 200 μM GPC 285937 or Mtx-mdbt-Dex. Clones visibly grew better on media containing GPC 285937 (e.g., page 129; Figure 7). The specification asserts that CPC 285937 has significantly better solubility and membrane permeability (e.g., page 75, 2nd paragraph). Applicant has not provided any direct evidence that GPC 285937 has unexpected properties relative to the closest prior art compound, which is compound 1p of Keenan et al.

The properties of better solubility and membrane permeability are not unexpected. Harris ("Introduction to Biotechnical and Biomedical Applications of Poly(Ethylene Glycol)." Poly(Ethylene Glycol) Chemistry: Biochemical and Biomedical Applications. Ed. J. Milton Harris. Plenum Press, New York, 1992. 1-14.) teaches that covalently linked PEG will solubilize other molecules and move molecules across cell membranes (e.g., page 3, items #13 and #18). Thus, it is not unexpected that GPC 285937 has greater solubility and membrane permeability compared to Mtx-mdbt-Dex.

Even if the results observed in the specification are unexpected, they are not commensurate in scope with the claimed invention. The properties of improved solubility and

permeability all relate to the ability to deliver the hybrid ligand to the cell by including the compound in a yeast cell growth medium, either liquid or solid. The claims do not contain a step of adding the hybrid ligand to a medium for the growth of yeast cells. The prior art teaches that hybrid ligands can be delivered to cells by any method known in the art, including methods such as electroporation (Mehta et al, page 15, lines 16-18; Liu et al, column 7, lines 3-15), which are not dependent upon solubility in culture media and membrane permeability of the compound. If the results are unexpected, Applicant has shown only that the linker of the formula $(CH_2\text{-}O\text{-}CH_2)_n$, where n=3 has improved properties. The claims are drawn to a genus of linkers. For example, Keenan et al (of record) teaches that lengthening the PEG linker results in reduced activity (e.g., $(\text{polyethylene glycol})_3 > (\text{polyethylene glycol})_4 >> (\text{polyethylene glycol})_5$). Applicant has not demonstrated that linkers of the formula $(CH_2\text{-}O\text{-}CH_2)_n$, where n=25 would have the proposed unexpected properties. Thus, Applicant has not provided evidence that the unexpected results occur over the entire claimed genus of R1-Y-R2 for the claimed method conditions.

At the paragraph bridging pages 22-23, the response asserts that Keenan's data clearly show that the hydrophobic linkers work well in the Keenan assay, while the PEG linker works very "poorly." It is unclear how the data is being interpreted to arrive at this conclusion. Keenan et al teach compounds 1h-1s, in which the linker moiety of parent compound 1d was varied (e.g., Table 1). Each compound was tested in a transient transfection assay, similar to the claimed invention. These results are shown in the column labeled "transient" in Table 1, where the percentage is the percent activity relative to the parent compound, 1d. For example, compound 1p had 85% activity as compared to 1d. Of the other 12 compounds tested, only 4 had higher

activity than compound 1p. The assertion that the PEG linker works "very poorly" is not supported by the empirical data provided by Keenan et al.

The response asserts that the PEG linker actually increases the cellular uptake of the hybrid ligands, in contrast to the teachings of Keenan et al which demonstrate "poor" results. This is not found persuasive, as Keenan et al are testing the exact same linker as tested in the present specification. Compounds with the same structure must have the same activity. The differences in result may be attributed to other factors such as the choice of R1 and R2, the choice of cell type (mammalian vs. yeast), and/or choice of fusion proteins. While nothing in Keenan et al suggests that the PEG linker will not work in a transient transfection assay, this variability provides evidence that Applicant has not demonstrated unexpected properties of the hybrid ligand over the claimed genus.

The response asserts that solubility is not the only reason a person skilled in the art would have been dissuaded from using a PEG linker as recited in the claims. The response points to the last line of page 1313 to page 1314 and asserts that "flexibility of a $(PEG)_5$ linker and a PEG-like linker compared to those of some non-PEG variants may account for the 'dramatic difference in activities.'" The sentence of the last line of page 1313 to page 1314 of Keenan et al states the following: "The greater flexibility of the five-atom linker of **1o** relative to the five-atom bisamide linker of **1n** may account for the dramatic difference in activities of these two compounds." Neither compound 1o nor 1n contains a PEG linker. Thus, this statement does not provide evidence that one would have been dissuaded from using a PEG linker as recited in the claims. The implication of less flexibility does not weigh heavily as a factor in determining the function

of the PEG linker compounds, because Keenan et al test each of the compounds in a transient transfection assay and provide the results in Table 1. These results are discussed above.

The response asserts that page 10622 of Amara provides further evidence of the lack of desirability of long linkers in that activity increased with shorter linkers. The response asserts that the invention is directed to very long linkers of 2-25 repeats. Keenan et al teach that the $(PEG)_3$, $(PEG)_4$ and $(PEG)_5$ linkers are functional in the context of the claimed assay. As discussed above the decreased efficacy of longer linkers taught by Keenan et al provides evidence that the proposed unexpected properties of GPC 285937 cannot be predictably extended to the other members of the genus. Further evidence is presented by Amara.

The response asserts that these arguments are applicable to all other obviousness rejections made by the Examiner. These arguments are not found persuasive for the reasons set forth above.

The response notes that Applicant does not understand how the Examiner can characterize the use of the hybrid molecule or linker of Keenan et al as predictable. Keenan et al teach the exact same linker as used by Applicant to demonstrate the proposed unexpected results. This linker was used in the context of an assay that reads on the claimed invention, for some claims (e.g., claim 28), with the only exception being that Keenan et al do not teach the step of identifying the nucleic acid sequence of the second chimeric gene encoding the candidate ligand binding domain that binds to the user-specified ligand R2, thereby identifying a polypeptide sequence that binds to a user-specified ligand. Otherwise, Keenan et al demonstrate that the hybrid ligands containing the $(PEG)_3$, $(PEG)_4$ and $(PEG)_5$ linkers are functional in the context of the claimed assay. Since the linker works in the assay taught by Keenan et al, there would be a

reasonable expectation of success in using either the entire hybrid ligand taught by Keenan et al or the linker taught by Keenan et al in the assay of Mehta et al. Perhaps Applicant is reviewing the stable transfection data, which demonstrate the lack of activity. It is noted that the claimed invention does not rely upon stable transfection, and Keenan et al teach that the hybrid ligands function in the transient transfection assay.

For these reasons, and the reasons made of record in the previous office actions, the rejections are maintained.

With respect to the rejection of claims 43-45 under 35 U.S.C. 103(a) as being unpatentable over Johnsson et al in view of Licitra et al and Bertozzi et al, as evidenced by Varshavsky et al, Applicant's arguments filed 11/2/2007 have been fully considered but they are not persuasive.

The response asserts that Licitra et al teach away from improving the linker in the hybrid ligand, because they suggest generating yeast strains that are more permeable without significantly affecting yeast viability. This is not found persuasive, because improving the invention by generating yeast strains that are more permeable does not preclude one from modifying or optimizing a hybrid ligand for use in the assay. As discussed by Applicant and noted in the prior art (Harris, J. Milton. "Introduction to Biotechnical and Biomedical Applications of Poly(Ethylene Glycol)." Poly(Ethylene Glycol) Chemistry: Biochemical and Biomedical Applications. Ed. J. Milton Harris. Plenum Press, New York, 1992. 1-14. See page 3, item #13) PEG is used to increase the solubility of covalently linked compounds. Thus, one would be motivated to increase the solubility of the compound regardless of any effect the modification may have on membrane permeability. Further, Applicant asserts that combining

Licitra with Bertozzi changes the principle operation of Licitra. This is not found persuasive, because the hybrid ligand retains the same function of bringing two proteins in close proximity. Moreover, the response asserts that one would not have been motivated to use the hybrid ligand containing a PEG linker, because the linker would be expected to by hydrophilic and thus not very membrane-permeable. This is not found persuasive, because PEG is known in the art to move molecules across cell membranes (Harris, J. Milton. "Introduction to Biotechnical and Biomedical Applications of Poly(Ethylene Glycol)." Poly(Ethylene Glycol) Chemistry: Biochemical and Biomedical Applications. Ed. J. Milton Harris. Plenum Press, New York, 1992. 1-14. See page 3, item #18). Furthermore, Keenan et al teach the effectiveness of compounds containing a PEG linker in the claimed assay format (e.g., Table 1), and thus the compounds would be expected to traverse the cell membrane. Applicant's arguments directed to the unexpected results are addressed above and are not persuasive for the same reasons.

For these reasons, and the reasons made of record in the previous office actions, the rejections are maintained.

With respect to the rejections made over the Liu reference, Applicant's arguments filed 11/2/2007 have been fully considered but they are not persuasive.

The response asserts that the PEG-linked hybrid ligands have unexpected membrane permeability. Further, the response asserts that the disclosure of electroporation and "other methods" supports Applicant's arguments. This is not found persuasive for the reasons set forth above. Even if the results are unexpected, they are not commensurate in scope with the claims. The claims do not require the addition of the GP 285937 compound to a yeast growth media.

The claims read upon the use of any method known in the art to introduce the hybrid ligand into any cell.

With respect to the rejections made over the Holt reference, Applicant's arguments filed 11/2/2007 have been fully considered but they are not persuasive.

The response asserts that the PEG-linker containing ligand has unexpected membrane permeability, which supports the non-obviousness of using the PEG linker in the claimed methods. This is not found persuasive for the reasons set forth above.

Further, the response asserts that Holt fails to render a species obvious where the prior art reference Holt only discloses a genus. Specifically, the response asserts that Holt teaches that the linker may be selected from a very broad range of structural types, and does not provide a relevant teaching as to which of the numerous types of linkers are preferred for any reason. This is not found persuasive, because Holt discloses species of ligands that meet the structural limitations of the claims. Holt et al teach the formation of heterodimers of immunophilin ligand moieties (e.g. FK506 and derivatives thereof) (e.g. page 1; page 6, lines 12-17). Holt et al exemplify structures linked by polyethylene linkers of the formula $(CH_2\text{-}O\text{-}CH_2)_n$, where n = 1, 2, 3, 4 (e.g. pages 14-15). Holt et al teach the use of the homodimeric ligands comprising the abovementioned linker where n = 2 or 3 in an cell-based transfection assay where a trimeric complex comprising the homodimeric ligand, a fusion protein comprising three copies of FKBP12 fused to a Gal4 DNA binding domain, and a fusion protein comprising three copies of FKBP12 fused to a VP16 activation domain was detected by the production of the reporter product, secreted alkaline phosphatase (e.g. pages 48-49). Holt et al teach it is within the skill of the art to use these linkers to optimize a particular ligand by routine experimentation. It would

have been obvious to substitute one known linker with another known linker in order to achieve the predictable result of linking two ligands for use in the claimed assay (e.g., page 48).

The response asserts that Holt does not actually specifically exemplify any heterodimeric ligand, and certainly not one with a PEG linker. This is not found persuasive, because Liu et al specifically teach heterodimeric ligands. Thus, Holt is not necessarily relied upon for this teaching. Furthermore, the claims do not all require a heterodimeric ligand. For example, claims 28-37, 39, 40 and 50-55 are readable upon hybrid ligands where R1 is identical to R2. In response to applicant's arguments against the references individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.,* 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986).

The response asserts that the Examiner has "presumed without any explanation or discussion that he level of skill for one of ordinary skill in the relevant art is 'high'." The response asserts that the level for one of ordinary skill in the relevant art is not high. The Examiner's statement that the level of skill in the art for one of ordinary skill is high is clearly substantiated by the teachings of each of the references cited in the rejections of record. These teachings are discussed in detail within the body of each of the rejections. The differences between the prior art teachings and the claimed invention relate to the use of a PEG linker, for example. These linkers were known in the art at the time the invention was made, and it was well within the skill of the ordinary skilled artisan to synthesize compounds containing these linkers (Keenan et al. Bioorg. Med. Chem. Vol. 6, pages 1309-1335, 1998, of record; Bertozzi et al. J. Org. Chem., Vol. 56, pages 4326-4329, 1991, cited as reference CC on the IDS filed

7/20/2005; Holt et al. WO 96/06097, cited as reference AD on the IDS filed 4/28/2003). A person of ordinary skill in the art would have been reasonably apprised of the use of a yeast-three hybrid assay (Mehta et al. WO 00/07018; Liu et al. US Patent No. 5,928,868). One would have been reasonably expected to have been able to modify the hybrid ligands used in three-hybrid assays to modify the linker. Keenan et al teach the construction of a series of hybrid ligands where either the R1/R2 moieties are varied or the linker moiety is varied. Furthermore, Holt et al teach that multimerizers vary somewhat in their observed activity, depending upon the particular chimeric proteins and other components of the system and recommend that the practitioner select multimerizers based upon their performance in the particular system of interest (e.g. page 48 lines 26-30). Given the broad base of knowledge in this area that is accessible to the ordinary skilled artisan, this changes would be considered modifications that are routine in the art relative to the level of skill of the ordinary skilled artisan. Thus, the above rejections are based upon what a person of ordinary skill would have known at the time of the invention, and on what a person would have reasonably expected to have been able to do in view of that knowledge.

For these reasons, and the reasons made of record in the previous office actions, the rejections are maintained.

Conclusion

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Jennifer Dunston whose telephone number is 571-272-2916. The examiner can normally be reached on M-F, 9 am to 5 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Joseph Woitach can be reached at 571-272-0739. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

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